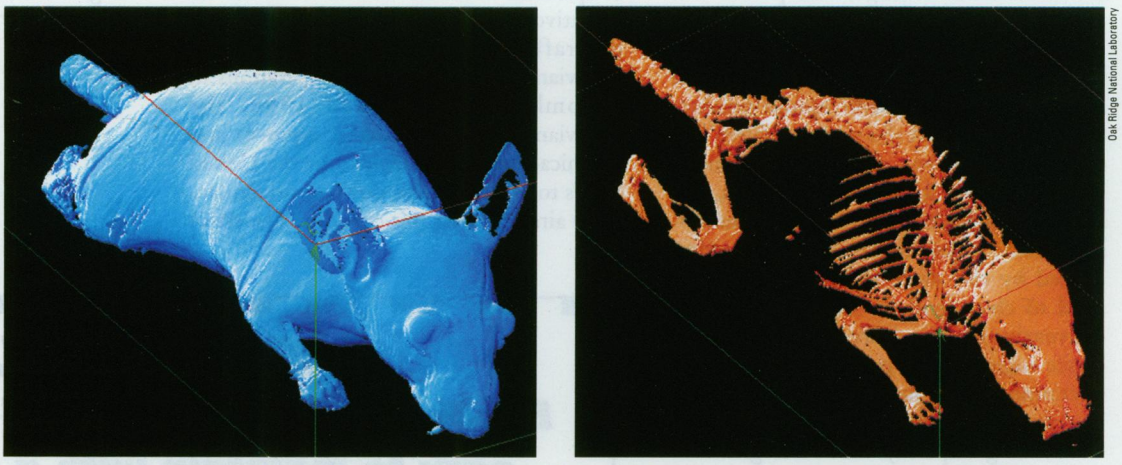
MicroCAT & Mouse

A new high-resolution X-ray computed tomography (CT) system developed by a team of researchers at the Department of Energy's Oak Ridge National Laboratory (ORNL) gives scientists a new option for examining soft tissue and skeletal details of mice and other small laboratory animals. Until now, researchers have had to follow visible genetic markers, conduct physical examinations, and dissect specimens to piece together the mysteries of genetic mutations. The new system, called MicroCAT, is set to change all that.

MicroCAT produces three-dimensional images with 20 times the resolution available with other medical imaging systems for humans. It also uses computer software to automatically analyze the reconstructed images of each specimen, eliminating the hours of tedious dissection once necessary to study genetic markers and experimental changes in mice.



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Same mouse, different day. Each whole-mouse data set can be manipulated to produce a variety of images. The images above represent the external surface (left) and the skeletal surface (right).

Because researchers won't need to dissect the mice, they can scan the same mouse at intervals over a period of weeks or months and thus track the development of a particular mutation over time. "This means we can survey many offspring of mutagenized mice for organ or skeletal abnormalities and for changes that occur as a mouse ages or is exposed to different environmental conditions, and then still breed for genetic analysis," says Dabney Johnson, a genetics researcher in the ORNL's life sciences division.

Why MicroCAT?

If anyone understands the importance of efficiently studying large groups of mice, Johnson does. The Mouse House at the ORNL is home to the world's largest colony of research mice—approximately 70,000 of them, all told. The Mouse House was established during the early days of atomic energy research soon after World War II, when ORNL scientists first began to research the biological effects of radiation exposure on mammals over generations. Today, the Mouse House boasts a stock of mutant mice with chemically induced deletions in their DNA representing approximately 400 mutant strains. This mutant collection is used to help scientists analyze gene function and identify mouse models of human genetic diseases. With so many different mice to study, Johnson and her colleagues sought a tool that would help them quickly and cost-effectively screen the residents of the mouse colony.

Thus began their collaboration with Michael Paulus, an electrical engineer in the ORNL's instrumentation and controls division. Paulus began to look at ways to automate and streamline this analysis. With fellow electrical engineer Hamed Sari-Sarraf and a team of researchers and experts from several divisions within the ORNL, he developed the MicroCAT system.

According to Paulus, studying mice offers great learning opportunities because the mouse genome is similar to the human genome. "If you can learn the function of a mouse gene," he says, "there is a very good chance you can learn that of the human gene." Biologists go through an extensive screening process to find a mouse that manifests a particular mutation. "Only 1 in 500 is going to have an expression of that [mutation]," says Paulus. Such screening is typically done by hand; researchers conduct physical exams to look for external markers, and dissect mouse after mouse to examine each animal's internal structure and verify mutations. This laborious undertaking is as expensive as it is time-consuming, so the goal was to automate the screening process and let the computer flag the images in certain mice that indicate a change or mutation.

Currently, the MicroCAT system can scan a mouse and gather necessary data in seven minutes. Says Paulus, "Once you have a 3-D map, the computer will identify images that may point to potential phenotypes. MicroCAT's detectors have intrinsic resolutions of less than 0.05 mm, enabling the system to produce reconstructed images with spatial resolutions of less than 0.05 mm." That means researchers can study mouse skeletons and organs with the same relative accuracy available to physicians studying human physiology with conventional medical CT systems, which have spatial resolutions on the order of 1–2 mm.

The MicroCAT system is a microscopic X-ray CT system, one of several imaging technologies available today. Others include magnetic resonance imaging (MRI), microscopic positron emission tomography, and microscopic single photon emission computer tomography. Still, these systems are quite expensive—for instance, MRI machines cost between $500,000 and $1 million—and are not widely available. The MicroCAT's high resolution and projected cost—approximately $150,000—make it an attractive choice. "This should be the least expensive of all the modalities," says Paulus.

Microscopic Mice

In the MicroCAT system, the mouse undergoes general anesthesia and enters the cavity of the scanner. The animal remains stationary while the scanner rotates slowly around it, stopping at discrete steps as small as a quarter of a degree to record a series of "glimpses." The resulting parallel projections, or cross-sections, are reconstructed via a computer program to generate the final 3-D image, which measures approximately 1.5 inches long and 1.5 inches in diameter. Paulus offers this analogy: "Imagine that you're walking around an object and taking hundreds of different one-dimensional X-ray images of it. If you project each of the images back over the image plane, you can construct a two-dimensional image, or 'slice,' of the object. Then you stack up all the slices to make a 3-D image." Although MicroCAT's method of computing the image doesn't really differ from that used in standard CT machines, MicroCAT does provide a higher resolution than that available with current CT technology, and is thus ideal for use on small animals such as mice.

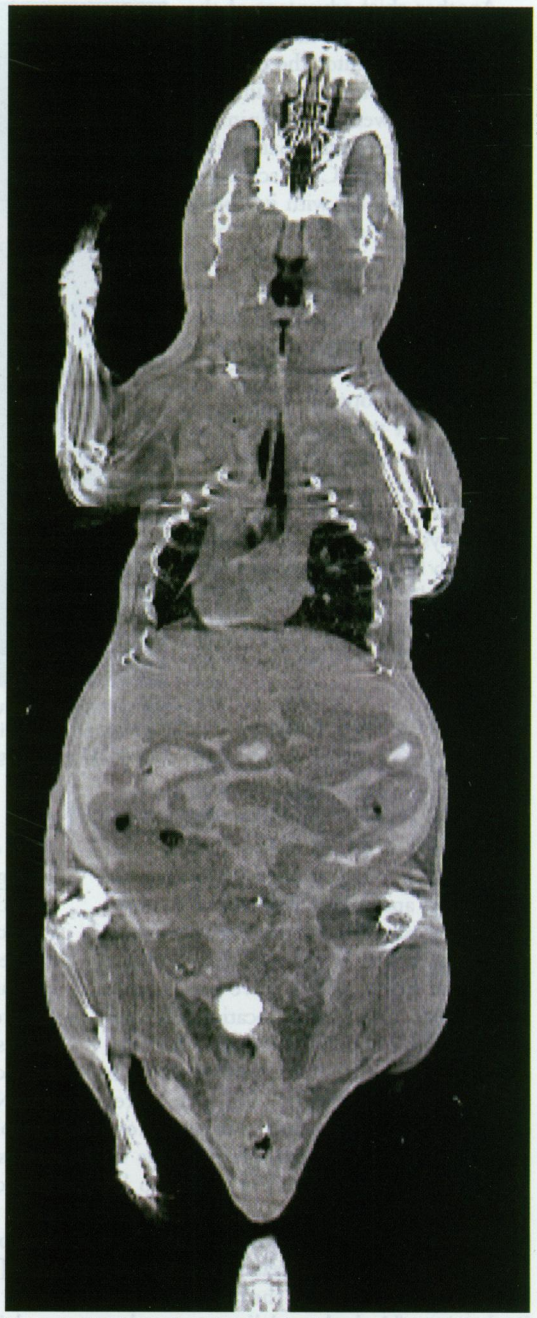
Paulus, Sari-Sarraf, and their team are perfecting two versions of the MicroCAT scanner. The first version uses a digital mammography detector with intrinsic resolution of less than 0.05 mm. While the detector completes the micro-CT scan in seven minutes, it can also be used to make a traditional X-ray image in less than a second.

In the second version, a unique energy-sensitive detector measures both the position and energy of each X ray. Because different materials in the body have different energy-dependent attenuation characteristics, MicroCAT's energy-dependent detector should produce a better quantitative measurement of the substance in the image plain. According to Paulus, incorporating X-ray energy information in the data set yields greater sensitivity to small variations in tissue density.

Another advantage to the second version is that researchers can conduct nuclear medicine research using the same scan, with MicroCAT producing images at a finer resolution than traditional imaging techniques. "The nuclear medicine data tell biologists about metabolic activity in the mouse, while the X-ray data provide high-resolution structural information," Paulus says. So far, the researchers have employed a single-pixel, energy-dependent detector to gather images. Work is now underway to develop a multielement detector array and the integrated circuitry associated with it.

The scanning portion of the MicroCAT technology is but one part of the system. The other parts, image reconstruction and computer-assisted data analysis, are performed after the raw data are recorded. Image reconstruction takes about 20 seconds per slide, according to Paulus. For a whole animal scan, as many as 2,000 slices may be reconstructed, although data are typically compressed into about 400 slices. A whole-mouse data set can be as large as 2–3 gigabytes and can take several hours to reconstruct, so the team often uses more than one computer to perform the reconstruction. What's novel about the MicroCAT system is that the computer connected to the scanning device automatically analyzes and interprets all the measurements that have been taken, and indicates which mice exhibit the problem or mutation in question, says Sari-Sarraf.

"The scanner is a step in the right direction," says Sari-Sarraf, "but you haven't really cut down on the work of the biologist. You still have this daunting task of going through volumes and volumes of images." But with a computer to automate the screening process and differentiate between what is normal and abnormal (for example, to identify that 4 or 5 out of the last 200 mice have a particular characteristic), Sari-Sarraf explains that the work is cut by 80–90%. It takes approximately 20–30 minutes to finalize a complete analysis at high resolutions. The image analysis software is still under development and should be complete by the end of 1999.



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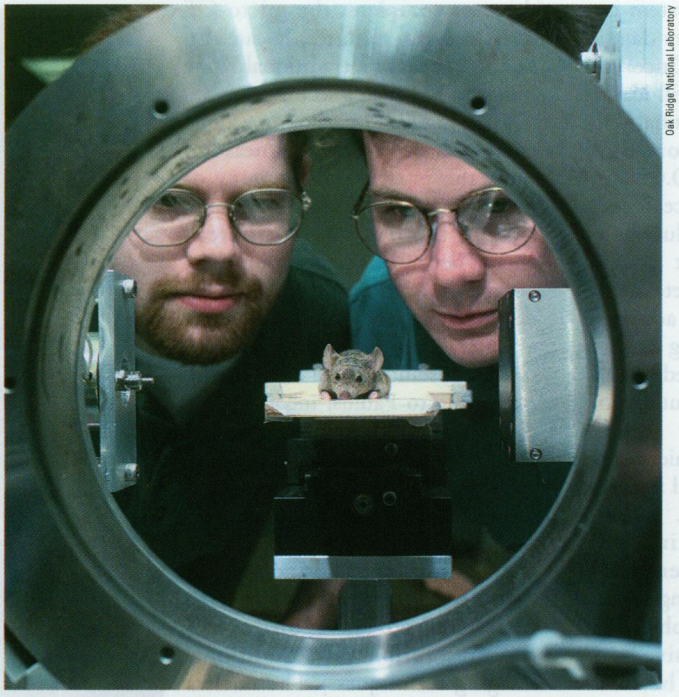
Deep in the bowels of mice. In this coronal image of a normal mouse, abdominal detail is enhanced using a nonionic iodine contrast agent.

Another chief advantage of the MicroCAT system is that it allows researchers to monitor the development of genetic mutations in mice over time, as well as the animals' response to treatments. Sari-Sarraf believes the MicroCAT scanning technology, coupled with its ability to provide automatic phenotype identification, will eventually help researchers quantify disease information and therapy for human benefit. Pharmaceuticals are one potential application, he says. "Any . . . pharmaceutical developed to address [a] disease is first tested on mice," he says. "With this system, you can track the mouse to see how effective a pharmaceutical treatment is." Paulus says the MicroCAT system could also be used to advance cancer research. It would be ideal to "screen for tumors and track tumor regression with [different] therapies," he says.



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All systems go. Kevin Behel (left), a graduate student from the University of Tennessee, and Mike Paulus (right) of ORNL look on as a mouse enters the MicroCAT scanning chamber.

MicroCAT's Future Lives

More work and future refinements lie ahead, though. "We're still in the initial stages," concedes Sari-Sarraf, who adds that the team has already used the new technology to study mutations in the kidneys and spleen. MicroCAT is a useful tool, he says, for imaging organs such as the brain, which is well defined, and the lungs and heart, which have predictable sizes, shapes, and relative locations. Images of the mouse's abdominal area are definitely more problematic. One possible solution is contrast agents, which can give better definition to the boundaries of these organs. In future implementations of MicroCAT, nuclear tracers may also be employed to help researchers study the function of an organ as well its structure. "We hope to add a nuclear imaging capability to the system in the near future," Paulus says. "A dual modality system that offers fully registered functional and structural data sets would be of great value to biologists."

Still, Sari-Sarraf and Paulus agree that for soft tissues, MRI provides superior images. "The MicroCAT's strength is skeletal imaging—anything with a strong density differential. MRI is better for soft tissue. That's where the two separate," says Paulus.

According to Robert Maronpot, chief of the NIEHS Laboratory of Experimental Pathology in the Environmental Toxicology Division, the chief concern about MicroCAT's effectiveness is how well it scans not just hard bone tissue, but also the marrow and the joints. "It depends on how well [the system] visualizes cartilage. If it [does this well], that could be a plus when studying diseases like arthritis," he says.

Maronpot agrees that the system's high resolution of 50 microns is a plus. However, in his laboratory, he and his colleagues often study individual cells ranging in size from 20 to 30 microns; only occasionally does he study cells, such as nerve cells, that approach 50 microns in size. "Still, 50 microns is good," Maronpot says. MRI easily yields a 50-micron resolution and, he adds, "we can push the envelope to get down to 20 microns."

Maronpot cautions that the MicroCAT system's high level of sophistication may hinder the average researcher from using it. He worries that most potential users may never be able to master the system if it's too complicated to use. Often, he says, systems such as this require "a constellation of different types of expertise, or seven or eight different experts" to operate the system and interpret and manipulate the data. Paulus counters that an important objective of the MicroCAT program is to build a user-friendly system that can be operated by a minimally trained animal care technician or student. Says Paulus, "The scanner uses a Windows-based graphical user interface and stores frequently used protocols for easy retrieval. Anyone familiar with Microsoft or Macintosh Windows-based programs can quickly learn to use MicroCAT."

Despite these concerns, Maronpot admits that MicroCAT's time has come. "There certainly is a need for it," he says, possibly even in a study he himself is conducting on a group of rats suffering from bone infarction, a condition caused by an interruption in the blood supply that leaves certain areas of the bone dead. According to Paulus, his team recently used MicroCAT to image a rat knee and the scanner clearly showed the skeletal tissue and cartilage, which means the system should be useful in studying diseases such as arthritis. Several biologists from around the country have already called Paulus to request scans of their research specimens.

Paulus's team has been testing and perfecting the MicroCAT system for about a year. They expect to deliver a full system to the Mouse House by the end of this year. Soon, Paulus hopes, the system will be available to anyone who wants to buy one.

Jennifer F. Medlin

Suggested Reading

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